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Induction of thromboxane synthase and prostaglandin endoperoxide synthase mRNAs in human erythroleukemia cells by phorbol ester

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The effects of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) on the mRNA levels of two enzymes, thromboxane synthase (TXS) and prostaglandin endoperoxide synthase (PES), responsible for the synthesis of thromboxane A_2 from arachidonic acid, were studied in human erythroleukemia (HEL) cells by RNA blot analysis. TPA induced both TXS and PES mRNAs in HEL cells in a dose-dependent manner at 36 h. The half-maximal and maximal effects for the induction of both mRNAs were at approximately 3×10^{-9} M and at 10^{-8} M, respectively. TXS and PES mRNA levels increased in a time-dependent fashion by TPA, and reached to 7- and 3.5-fold of the control, respectively after 48 h of TPA treatment. These results suggest that expression of TXS and PES genes in HEL cells were simultaneously stimulated by TPA.

Thromboxane synthase; Prostaglandin endoperoxide synthase; 12-*O*-Tetradecanoyl-phorbol-13-acetate

1. INTRODUCTION

Thromboxane (TX) A_2 is a potent stimulator of platelet aggregation and constrictor of smooth muscles [1,2], and is regarded as a mediator in myocardial infarction, atherosclerosis, and bronchial asthma [3]. Synthesis of TXA_2 from arachidonic acid is catalyzed by two enzymes, prostaglandin endoperoxide synthase (PES) and TX synthase (TXS) [4,5]. Recently, the cloning of cDNA coding for PES has been reported [6–10], and the regulation of PES gene expression by several factors, including TPA, platelet-derived growth factor, etc., has been studied extensively [11]. More recently, we and Ohashi et al. reported the cloning cDNA encoding human platelet [12] and lung TXS [13], respectively. Furthermore, TXS mRNA was found to be expressed in platelets and human erythroleukemia (HEL) cells [12]. However, little is known about the regulation of gene expression of TXS and the relationship between the expression of PES and TXS genes.

HEL cells exhibit some erythroid features including the presence of hemoglobin, but they also possess the markers corresponding to megakaryocyte/platelet surface glycoproteins, and platelet α-granule proteins, which are induced by the treatment of TPA [14]. TPA also induces morphological, functional, and biochemical properties in HEL cells that are characteristic for megakaryocytic cells [14] and macrophage-like cells [15]. Thus, the HEL cell line provides a unique in vitro model system for studying the molecular events involved in TXS gene expression.

In this report, we demonstrate that TPA increases the levels of TXS mRNA in a dose- and time-dependent fashion in HEL cells, which is similar to that of PES mRNA by TPA.

Abbreviations: TXS, thromboxane synthase; PES, prostaglandin endoperoxide synthase; HEL cells, human erythroleukemia cells; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

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2. MATERIALS AND METHODS

2.1. *Materials*

HEL cells were kindly provided by Dr. S. Narumiya (Kyoto University). RPMI 1640 medium was obtained from Gibco/BRL, fetal bovine serum from Flow laboratories, 12-*O*-tetradecanoyl-phorbol-13-acetate from Sigma, and [α-^{32}P]dCTP (110 TBq/mmol) from Amersham. Other reagents were described previously [16].

2.2. *Cell culture*

HEL cells [17] were maintained in suspension in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 5% CO_2 in air. Cultures were passaged 2–3 times a week to maintain a log-phase growth. For TPA treatments, cells were placed in fresh medium at 1.2×10^7 cells/150 mm dish and were cultured for an additional 18 h. TPA was then added to the culture medium from a stock solution prepared in ethanol. Control cells received the same amount of ethanol (0.05%). Cell viability at the cell culture was estimated by Trypan blue exclusion to be more than 95%.

2.3. *RNA isolation and RNA blot hybridization analysis*

Total RNA was isolated according to the acid guanidinium thiocyanate procedure [18]. Adherent HEL cells in 150-mm dishes were lysed with 4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5%

N-lauroylsarcosine, and 0.1 M 2-mercaptoethanol. Non-adherent cells were collected by centrifugation and lysed with the same solution. The lysate was passed through an 18-gauge needle three times to shear chromosomal DNA. The RNA was extracted in sodium acetate (pH 4.0) and phenol–chloroform and precipitated in isopropanol. Total RNA (20 μg) was run on a 1.2% agarose gel according to the procedure described by Miyakoshi et al. [19] and transferred to a nylon membrane. The cDNA probes used for the analyses of the two mRNAs were as follows: for PES mRNA, the 1.7 kb *Eco*RI fragment of the PES cDNA clone (λHEPES 13) isolated from a cDNA library of human endothelial cells (unpublished data); for TXS mRNA, the 1.2 kb fragment derived from pHPTXS6 [12]. The probes were labeled by the random primer labeling method [20]; specific radioactivities were 1.0–1.5 × 10^9 cpm/μg. The mRNA levels were calculated on the basis of hybridization signals measured by a Fujix Bio-image analyzer BAS 2000 (Fuji Photo Film Co., Tokyo). The relative intensity measured in the hybridization signal was linear within the ranges of 5–30 μg of total RNA.

3. RESULTS AND DISCUSSION

With the use of the cDNA clone for human platelet TXS, we examined the effect of TPA on TXS mRNA levels in the HEL cell line by RNA blot analysis. At first, to test the dose-dependent effect on TXS mRNA levels, HEL cells were incubated with various concentrations of TPA for 36 h. The total RNA was isolated and subjected to quantitation of relative levels of TXS mRNA. As shown in Fig. 1A, TPA increased TXS mRNA levels half-maximally at approximately 3 × 10^{-9} M and maximally at 10^{-8} M. Above 10^{-7} M TXS mRNA levels decreased with an increasing concentration of TPA. We also treated the dose-dependency to TPA in PES mRNA levels by using its cDNA clone, because PES supplies the substrate of TXS, prostaglandin H_2. A similar sensitivity to TPA was observed in the induction of PES mRNA. This induction was one-half maximal at about 3 × 10^{-9} M and maximal at 10^{-8} M. PES mRNA levels also decreased at concentrations over 10^{-7} M TPA (Fig. 1B). Interestingly, the dose-dependent curves of the increase in both TXS and

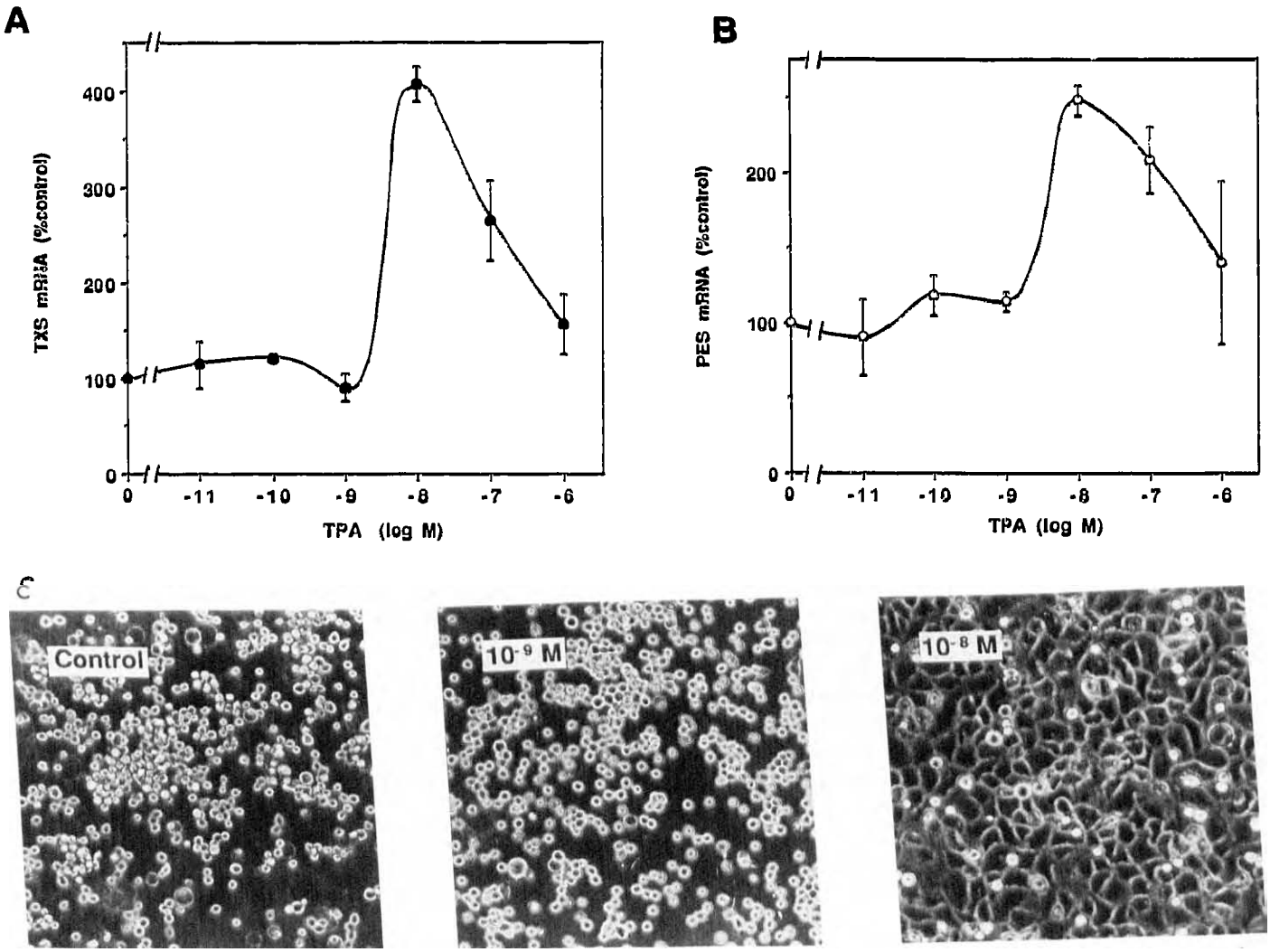

Fig. 1. Dose-dependent effects of TPA on TXS and PES mRNA levels. Total RNA was prepared from HEL cells treated with various concentrations of TPA for 36 h, and RNA blot hybridization analysis was performed as described in Materials and Methods. The mRNA levels of TXS (A) and PES (B) were determined by the Image Analyzer, with the zero time level taken as 100%. Data are the mean ± S.D. of quadruplicate determinations. (C) Induction of adherence of HEL cells at 36 h after addition of various concentrations of TPA (magnification × 100).

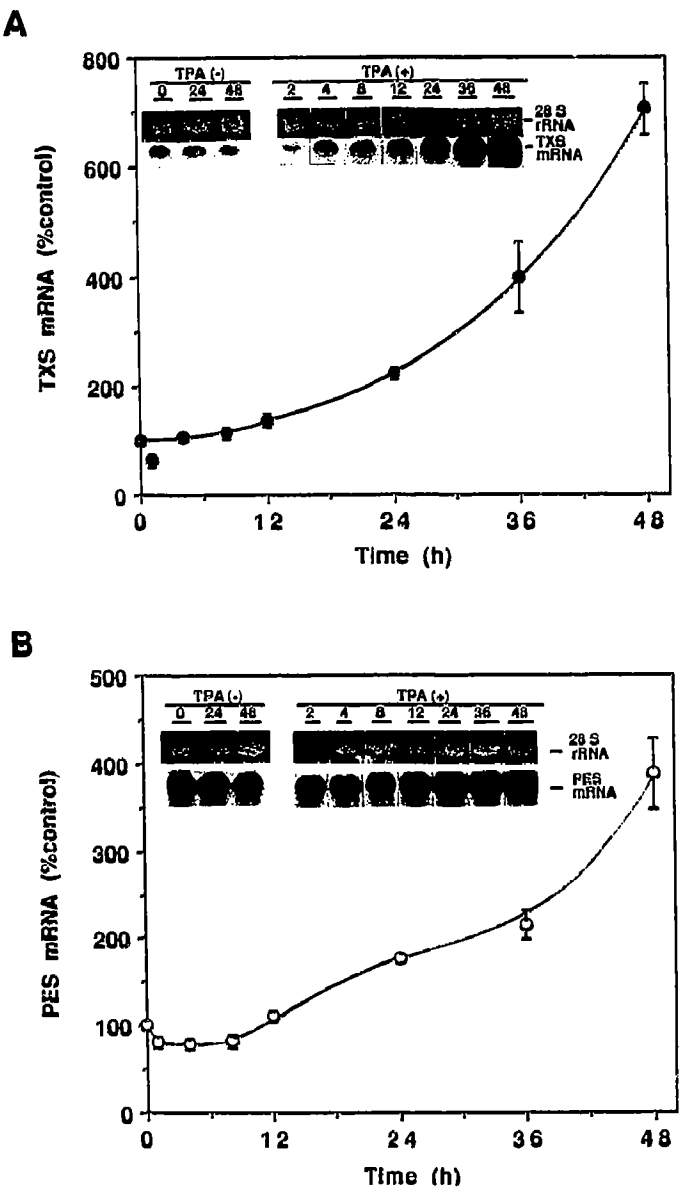

Fig. 2. Time-course of changes in TXS and PES mRNA levels in HEL cells after addition of TPA. Experimental details are described in Materials and Methods. Total RNA analyzed was prepared from HEL cells immediately before (0 h) or at the indicated times after addition of TPA (10^{-8} M). The mRNA levels of TXS (A) and PES (B) were determined by the image analyzer with the zero time level taken as 100%. Data are the mean ± S.D. of quadruplicate determinations. Inset, representative RNA blot analysis; TPA (+) and TPA (−) indicate total RNA isolated from cells incubated with and without TPA, respectively, at indicated times. Ethidium bromide staining of the 28 S ribosomal RNA corresponding to each lane is shown.

PES mRNAs by TPA were similar to that of induction of adherence in HEL cells. The critical concentration of the induction of adherence was also between 10^{-9} and 10^{-8} M. At TPA concentrations lower than 10^{-9} M, HEL cells behaved like the control culture even after 36 h of incubation. In contrast, above a concentration of 10^{-8} M, HEL cells were dramatically changed to adherent cells (Fig. 1C, [15]). These observations suggest that the stimulation of expression of PES and TXS genes by TPA in HEL cells might be associated with the induction of morphological change of the cells.

To analyze the time-course for induction of TXS and PES mRNA levels, HEL cells were incubated in the presence of 10^{-8} M TPA for various periods. Total RNA was isolated and analyzed by RNA blot hybridizaton. TXS mRNA levels gradually increased and led to an elevation of about 2-fold of the control after 24 h of the treatment. After 48-h incubation with TPA, the mRNA levels reached 7-fold of that of the control. In contrast, there was no apparent change in TXS mRNA levels in the absence of TPA (Fig. 2A). The results of PES mRNA induction shown in Fig. 2B indicate that the content of PES mRNA was also elevated time-dependently 1.8-fold after 24 h of TPA incubation. This observation is consistent with the results reported by Funk et al. [10]. Furthermore, PES mRNA levels increased 3.5-fold above control values 48 h following the TPA addition. These results suggest that the expression of TXS gene is simultaneously regulated with the PES gene by TPA in HEL cells. Lag periods of about 10 h occured before an increase in both mRNA levels by TPA were seen. The relatively late induction suggests that the effect of TPA might be indirect and require an ongoing synthesis. Although c-*jun* and c-*fos* mRNAs are induced rapidly and transiently by TPA in most cells [21], c-*jun* mRNA is greatly stabilized [22], and c-*fos* mRNA remains elevated throughout the TPA-induced differentiation in the megakaryocytoid leukemia line, K562 [23]. By analogy, the late induction of TXS and PES mRNAs by TPA might be due to the prolonged induction of c-*jun* and c-*fos* mRNAs in HEL cells.

This report provides the first evidence indicating that thromboxane synthase is regulated at the mRNA level by TPA. Furthermore, TPA induced both TXS and PES mRNAs in HEL cells in a similar dose- and time-dependent manner, suggesting that the expression of both genes is simultaneously regulated by TPA. At present, the localization of TPA-responsive element (TRE) in the human TXS gene is not yet known, and it is also not known whether or not AP-1 and Fos proteins are involved in the induction of TXS mRNA in TPA-treated HEL cells. On the other hand, PES gene, the expression of which is simultaneously stimulated with that of the TXS gene by TPA, has four putative TREs [24]. To elucidate the molecular mechanisms of TPA-mediated induction of TXS mRNA, the analysis of the 5′ regulatory sequence of the human TXS gene is now in progress.

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